REMARKS

Claims 20, 22-31 are pending in the present application, with claims 23-28 being withdrawn. Claim 21 has been cancelled. Claims 29-31 have been added. Support for new claims 29-31 may be found on page 26, lines 16-25, and page 34, lines 22-25.

Rejections under 35 U.S.C. §103

Claims 20 and 21 have been rejected under 35 U.S.C. §103 as being obvious over Sagawa et al. combined with Nordstrom et al. Applicants traverse this rejection and withdrawal thereof is respectfully requested. The present invention is drawn to a method for isolating a desired gene which encodes a protein lethal or harmful to the host by

(i) inserting DNA fragments containing the desired gene into a plasmid vector(s) comprising a promoter sequence to control expression of a desired gene, wherein the promoter sequence is recognized by an RNA polymerase derived from SP6 phage, and a replication origin for increasing a copy number by induction with an exogenous factor, wherein the replication origin contains a lac promoter and an RNAII region;

(ii) transforming host cells with said vector(s); and

(iii) selecting host cells containing said desired gene.

Thus, the present invention, contemplates and permits the cloning of genes which are harmful to the host, for example,

restriction endonucleases which are otherwise lethal to the host cell. As shown in the Examples and described on page 26, lines 16-25, the present inventors have demonstrated the ability to clone and express the Nsp7524 III restriction endonuclease without the co-presence of a corresponding modification enzyme gene. These results demonstrate the ability to clone lethal genes using the plasmid vector system of the present invention. Claim 1 recites that the gene is "harmful or lethal" to the host.

Cloning of harmful genes, such as endonucleases, has proven to be particularly difficult, due to the lethality of the gene products to the host cell. As discussed on page 5 of the specification, cloning of restriction endonucleases has been reported as being impossible due to the incomplete modification system in the host, resulting in host death. However, the present invention achieved this "impossible" goal and there is no suggestion of the present invention in Sagawa et al. combined with Nordstrom et al.

Sagawa et al. discloses an expression system having an SP6 promoter, which can be regulated with the non-indigenous SP6 RNA polymerase, and which is controlled by the inducible *lac* promoter.

However, with the expression system of Sagawa et al., when the host is *E. coli*, the inserted desired gene located downstream of the SP6 promoter is expressed in small amounts, even under non-inductive conditions because the SP6 promoter is weakly recognized by RNA polymerase from *E. coli*. As a result, if the expression

system of Sagawa et al. cannot be used if the inserted gene encodes a protein harmful or lethal to the *E. coli* host.

There is no disclosure in Sagawa et al. of the problems associated with cloning harmful or lethal genes or how to overcome those problems. Sagawa et al. do suggest that the basal expression level of the gene may be reduced using a "low-copy-number plasmid vector." However, there is no disclosure regarding cloning harmful genes and use of a low-copy-number plasmid vector is, in fact, the exact opposite of the teachings of the secondary references.

The secondary reference of Nordstrom et al. is directed to the production of large quantities of proteins from cloned genes in bacteria when expression is desired. Runaway plasmids are used as one tool for massive DNA amplification in the secondary references. However, the secondary reference fails to suggest lowering the copy number to a non-lethal level to a host cell.

Thus, the concept of increasing copy number when expressing the desired gene is common to both the present invention and the secondary reference of Nordstrom et al. However the present invention couples the concept of increasing copy number during expression with the copy number being decreased to a non-lethal level in the non-inductive state. There is no suggestion of this second aspect of decreasing the copy number to a non-lethal level in either the primary or secondary reference. In fact, the reduction in expression level taught by Nordstrom et al. would be insufficient

to allow the cloning of a restriction enzyme gene. As such, Nordstrom et al. fails to teach what is lacking from Sagawa et al. and the present invention is not achieved from the combined references.

Claim 22 has been rejected as being obvious over Sagawa et al. combined with Nordstrom et al. and Brooks et al. Further to the asserted teachings of Sagawa et al. and Nordstrom et al., discussed above, Brooks et al. is asserted to teach the toxicity of restriction endonucleases in host cells such as *E. coli* and the difficulty of cloning endonucleases. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Brooks et al. discloses a method of producing restriction endonuclease *Bam*HI by introducing the *Bam*HI modification system or methylase gene into a host cell, expressing the methylase gene to thereby protect the host cell from cleavage by the corresponding *Bam*HI restriction endonuclease and introducing the *Bam*HI restriction gene into the host. In addition, in columns 1-3, Brooks et al. teach that it is necessary to select a suitable host *E. coli* to carry out the cloning of the restriction-modification system gene.

However, in the method of Brooks et al., the co-expression of the methylase gene in the host cell is required for the expression of restriction enzyme without killing the host cell. Thus, with

Brooks et al. the co-expression of the methylase gene is required for the expression of the restriction endonuclease gene. Based on the teachings of Brooks et al., one skilled in the art would not be motivated to express a restriction endonuclease without the co-expression of a modification gene. In addition, Brooks et al. fails to teach or disclose transcription under the control of a promoter that is controlled through the copy number. One skilled in the art would not be motivated to combine Nordstrom et al. with Brooks et al. to express a gene that encodes a protein harmful to the host because Nordstrom et al. teach increasing copy number to over-express a protein encoded by the gene. Even if the references are combined the resulting teaching is a method of producing a restriction endonuclease on a large scale with the co-expression of the methylase gene by increasing the copy number of the restriction endonuclease gene. Thus, the teaching of the combined references is completely contrary to the method employed by the present invention. As such, the present invention of claim 22 is not obvious over Sagawa et al. combined with Nordstrom et al. and Brooks et al. and withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §112, second paragraph

Claims 20-22 have been rejected as being unclear for failing to recite active steps for the claimed method. Claim 20 has been

amended to recite active method steps. Withdrawal of the rejection is respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, PhD (Reg. No. 40,069) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

A marked-up version of the amended claims is attached hereto.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a two (2) months extension of time for filing a reply in connection with the present application, and the required fee of $400.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any

overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Marked-up version showing changes

Claim 21 has been cancelled.

Claim 20 has been amended as follows.

20. (Amended) A method for isolating a desired gene, ~~characterized in that~~ **which comprises**

(i) (a) cleaving DNA containing the desired gene with restriction endonucleases to generate DNA fragments, or

(b) creating a cassette library of DNA containing the desired gene, amplifying the DNA of the cassette library, and isolating the desired gene;

(ii) inserting said fragments of (a) or desired gene of (b) into a plasmid vector(s) ~~a plasmid vector~~ comprising a promoter sequence to control an expression of a desired gene, said promoter sequence being recognized by an RNA polymerase **derived from SP6 phage** ~~not inherent to a host~~, and a replication origin for increasing a copy number by induction with an exogenous factor**, said replication origin comprising lac promoter and RNAII region, wherein said desired gene is a restriction endonuclease gene and the plasmid vector comprises the restriction endonuclease gene without co-presence of a corresponding modification enzyme gene;**

(iii) transforming host cells with said vector(s); and

(iv) selecting host cells containing said desired gene.

New claims 29-31 have been added.